THE MATURATION PHASES IN SMILAX HERBACEA

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(WITH PLATES IV–VI)

The material for this paper was collected in the spring of 1909 in the vicinity of New Haven, Connecticut. Both staminate and pistillate flowers were obtained with a view to studying nuclear conditions in both sexes. Staminate flowers, gathered on May 14, supplied nearly all the stages desired, as the flower buds in each inflorescence exhibited varying degrees of development. The pistillate flowers, maturing more slowly, were fixed May 26 and June 2. The series obtained from this material was very incomplete, only a few stages of the prophase of the heterotypic division being procured. Traces of the megaspores were visible in some flowers, but in most cases their location was represented by a dark, irregular line which suggested crushing or imperfect fixation.

Various killing fluids were used, but only two proved of any value, namely Flemming's fluid (weaker solution) and Juel's fluid. Sections were cut 6μ in thickness and stained with Flemming's triple stain or Haidenhain's iron haematoxylin.

The maturation phases in the microsporangium

The earliest observations of the sporogenous tissue were made after the telophase of the last vegetative mitosis and before the differentiation of the tapetum. Excluding the outer layer of cells in this tissue, which eventually become tapetal, the remaining cells are virtually pollen mother cells, and after a slight increase in size are ready for the phenomena characterizing meiosis. The nuclei of the young spore mother cells show small chromatin bodies or granules of variable size scattered through the finely granular linin meshes. A distinct reticulum is not present. Often the chromatin bodies may be seen in pairs or groups of four, but their distribution is generally irregular and does not warrant a conclusion that pairing is their typical arrangement.

A multinucleolate condition is typical of the nuclei of these cells. The nucleoli are variable in number and size and often somewhat angular in outline; several small bodies appear attached to the nucleoli (figs. 2, 3), which resemble papillae and will be so designated during the following description. As late as diakinesis nucleoli have been observed with one or more of these papillae. In the material prepared with the triple stain the nucleoli of the heterotypic prophase show one or more glistening white spots; these were at first considered to be vacuoles, but there is also the possibility that they represent papillae viewed on the upper surface of the nucleoli. The microspore mother cell in the early prophase is sometimes uninucleolate, though more often provided with two large nucleoli. However, at synapsis there is never more than one large nucleolus, which is no longer angular and is usually provided with a single papilla.

In connection with the study of the microspore mother cells of the early prophase, observations were made on the somatic nuclei of the nucellus. The appearance of the chromatin bodies and the nucleoli in such a nucleus (fig. 27) agrees very closely with that given for the nuclei of the young spore mother cells.

The author believes the uninucleolate condition (fig. 5) to be the result of union of the nucleolar elements. Fig. 1 shows two nucleoli connected by a short, deeply stained strand, while fig. 2 shows two nucleoli in a later stage of fusion. The papillae described above are probably nucleolar fragments or very small nucleoli which are in the process of fusing with the larger nucleoli. Fusion of all the nucleolar matter apparently does not take place; as late as diakinesis small globular bodies are often found which are distinct from both the nucleolus and the chromosomes. The papillate condition of the nucleolus also persists until the nucleolus disappears at the metaphase.

GATES (14) describes parallel phenomena in the sporogenous cells of *Oenothera rubrinervis*. Here the nucleus is provided with one large nucleolus accompanied almost invariably by smaller nucleolar bodies. Fusion of these bodies takes place; the number present in later stages depends on the amount of fusion. This seems to vary. One large nucleolus is always present until the

disappearance of the nuclear membrane; there are also one or two small nucleolar bodies which remain through the metaphase of the heterotypic division and are sometimes observed on the homotypic spindle.

This papillate appearance of the nucleolus may be interpreted in quite another way, namely, as a process of chromatin budding from the nucleolus. CARDIFF (5) figures nucleoli with similar papillae in young spore mother cells, and suggests that a papilla may represent the beginning of a chromatin thread formed from the nucleolus. Miss NICHOLS (32), in a study of several species of *Sarracenia*, concludes that the nucleolus of the pollen mother cells elaborates the chromatin, and figures nucleoli with small bodies attached, which represent the chromatin emerging from the nucleolus. DARLING (7) describes the budding of chromosomes from the nucleolus in the prophase of the heterotypic division in *Acer Negundo*. In the somatic nuclei of the root tip of *Phaseolus*, WAGER (43) describes the nucleolus as being connected with suspending fibers along which the chromatin from the nucleolus passes. These fibers become much thickened with the accumulation of chromatin and finally break up into chromosomes. With the loss of chromatin the nucleolus shrinks, becomes detached from the chromosomes at the metaphase, and finally disappears. The conclusions of WAGER, however, are disputed by MARTINS MANO (26), who made a similar study of *Phaseolus* and concluded that the chromosomes are not of nucleolar origin. He advances this interpretation: at the telophase certain portions of the chromosomes are drawn out into threads which anastomose and form a chromatic reticulum. In the following prophase the reticulum gradually assumes the form of bands with connecting fibers; the bands contract and ultimately break up into chromosomes. The appearance that WAGER describes as "suspending fibers" MARTINS MANO interprets in another way. The nucleolus is formed independently from nucleolar substances, but in close contact with chromatin elements. When the perinucleolar vacuole is formed, there is a consequent repulsion of the nuclear reticulum; certain parts of it remain attached to the nucleolus by reason of the elasticity and viscosity of the chromosomes. If the nucleolus furnishes

any substance to the chromosomes it is not done by means of "suspending fibers."

Fig. 3 seems to show a condition like that described by DARLING and WAGER. There is a great similarity between the papillae attached to the nucleolus and the chromatin bodies lying free in the linin network. On the other hand, the presence of numerous darkly staining granules in the sporogenous and somatic nuclei and the presence of a nucleolus as late as diakinesis argue against the resolution of the nucleolus into chromatin bodies.

SYNAPSIS.—The term "synapsis" has been defined in two ways. Most botanists use the word to denote the period of maximum contraction of the chromatic elements in the prophase of the heterotypic division. Zoologists call this stage "synizesis" (McCLUNG **25,** JORDAN **20**) and assign the name "synapsis" to the period of approximation of parental chromosomes. GRÉGOIRE (**17**) defines synapsis as covering stages from leptonema to strepsinema. In this paper "synapsis" will be considered as synonymous with "synizesis."

The presynaptic phases in *Smilax herbacea* are apparently simple; the linin mesh contracts (fig. 6), drawing the chromatin bodies together into an increasingly close proximity. As there is no chromatic reticulum or pairing of thin filaments, the process resolves into the mutual approach of chromatic bodies. MIYAKE'S (**28**) description of chromatin behavior in *Lilium* corresponds closely to this account. During synapsis (fig. 7) the nucleolus is almost invariably at one side and projecting from the synaptic mass; delicate linin threads bridge the karyolymph and connect the chromatic ball with the nuclear membrane. For a time the granular nature of the chromatin is maintained; toward the end of synapsis, however, the chromatin becomes arranged in a much interwoven beaded filament (fig. 8).

Views in regard to synapsis and its importance range from those that discard it as of no significance or due to faulty technique (SCHAFFNER **37**) to those that assign to it the function of bringing together parental elements in closest union and mutual influence. LAWSON (**22**) denies the presence of any real contraction and states that the phase known as synapsis is due to an enlargement of the

nuclear cavity, the chromatic mass remaining the same size. In 1899 GUIGNARD (19) reported the absence of synapsis in *Naias major*. CARDIFF (5) has described this phase as the deferred culmination of fertilization. The later tendency is to admit its presence as a normal stage and to consider it only a time of great shortening and thickening of chromatic filaments (GRÉGOIRE 17, BERGHS 2, DAVIS 8). GRÉGOIRE in his discussion of synapsis admits that it is not a universal phenomenon. When it does occur, he believes it can have no rôle to play in the process of reduction, but is itself a result of certain nuclear activities. He further suggests that the appearance of synapsis may be emphasized by the growth of the nuclear cavity or by an artificial contraction caused by fixing reagents enhancing the natural contraction. GATES (16) states that it is evident many changes take place during synapsis, though there may be no interchange or influence between homologous chromosomes. He points out (15) that such influences may take place at any time during the sporophytic phase of the life cycle. ERNST (12) considers synapsis normal, otherwise a similar sensitiveness to fixing fluids ought to show in vegetative mitosis of corresponding stages. There is one possibility favoring artifact, namely, that the progressive stages of mitosis may be accompanied by chemical changes in the chromatic substance which cause different reactions to fixing fluids. It is difficult, however, to conceive of a chemical change occurring in a heterotypic prophase which would not also occur in a somatic prophase. Moreover, there is no experimental basis for this view, though NĚMEC (30) by microchemical tests demonstrated differences in the chromatin of resting and dividing nuclei.

The contraction of the nuclear contents is very striking in *Smilax herbacea* (cf. figs. 5, 6, 7), and is without doubt a normal condition. It is difficult to assign synapsis a rôle in *Smilax*. It is evident that the appearance of the nucleus after synapsis differs markedly from the preceding conditions; the chromatin elements pass into synapsis as distinct bodies and emerge in a homogeneous filament. Until more light is thrown upon this phase, we can only vaguely state that synapsis may facilitate the proper placing of the paired parental elements in the chromosomes and the chromo-

somes in the spireme. The chromosomes in *Smilax herbacea* never appear as definite units until the segmentation of the spireme.

POSTSYNAPSIS.—The much coiled filament of late synapsis emerges as a fairly thick thread slightly beaded (fig. 8), which later assumes a homogeneous character. The double nature of the spireme is early discernible (fig. 9). With the continued loosening of the knot the split is sometimes obliterated, but the spireme as it is distributed throughout the nuclear cavity is distinctly double; this separation of previously paired[1] chromatic elements does not appear simultaneously in all parts of the spireme (figs. 10, 11, 12). Shortening and thickening of the spireme proceeds as usual, followed by a sort of semi-segmentation of the double filament. At intervals along the spireme occur places where each longitudinal half is apparently constricted to a delicate thread (fig. 13). This appearance is due doubtless to incomplete transverse segmentation with subsequent pulling apart of the double segments; the attached portions thus are drawn out into fine threads. The bivalent chromosomes resulting from the completion of the transverse division of the spireme are long and slightly twisted about each other (figs. 14, 14*a*). Though not a typical strepsinema, this condition corresponds to strepsinema as described by GRÉGOIRE (17).

The shortening and thickening process continues, resulting in the characteristic diakinetic gemini, the univalent halves of which lie, indiscriminately (fig. 15), parallel, at right angles to each other, or in the form of V's or X's. Traces of linin threads can be seen attached to the ends of the chromosomes. The nucleolus is present at this stage (though not figured), but disappears before the metaphase. Small globular bodies scattered about among the gemini appear in many of the nuclei in addition to the nucleolus. These are probably small nucleolar bodies as previously described.

METAPHASE.—Many of the gemini retain the semblance of a V on the spindle (fig. 16), though occasionally the homologous chromosomes are oriented in a straight line. Intermediate stages occur between these two types of orientation. In many cases the chromosomes show a distinct splitting while at the equator; this is more marked as the chromosomes pass toward the poles, though

[1] Previous pairing is assumed to have taken place.

it does not become a complete fission (fig. 19). This is without doubt a genuine splitting preparatory for the homotypic mitosis.

As the first division is merely a separation of chromosomes, the true mitosis being deferred until the second division, it is not surprising that the fully formed chromosomes exhibit a tendency to fission, the normal consequence of a mature condition in meristematic cells, long before actual mitosis is permitted to take place. FARMER (13) expressed a similar idea when he said "with the inception of karyokinetic activity the spireme thread undergoes the longitudinal fission characteristic of ordinary somatic division, although the actual separation of these longitudinal halves is deferred until the next mitosis."

The separation of the chromosomes at the equator and their passage to the poles takes place in the usual manner. Frequently the chromosomes of one or more pairs separate and move away from the equator earlier than the majority (figs. 17, 18).

INTERKINESIS.—At the telophase the chromatic elements appear in the form of a spireme which is disposed about the periphery of the newly formed nuclear membrane. The daughter nuclei are usually elliptical, though sometimes they are slightly curved, presenting a concave surface toward the equatorial plate. MIYAKE (28) finds in *Lilium Martagon* a partial formation of a thread, but usually there is little change in the form of the chromosomes during interkinesis. In *Smilax herbacea* the split which was observed in the metaphase and anaphase, homotypic in nature, is sometimes faintly discernible, but usually lost to view. Vacuolation of the chromatin band, if it may be said to occur at all, is very slight. In fact, the transitory character of this phase does not call for extensive alveolization. We have here in reality a prophase of the homotypic division. GRÉGOIRE (17) describes the heterotypic division as a process intercalated in the prophase of the homotypic division.

Conditions reported during interkinesis vary in different plants. In *Oenothera gigas*, according to DAVIS (9), the daughter chromosomes maintain their form and distinctly show the homotypic split, thus giving an appearance similar to diakinesis. GATES (16), describing the same species, states that some of the chromosomes

pass through interkinesis in a compact condition, while others become vacuolate. In *Nephrodium molle* (YAMANOUCHI **45**) the chromosomes become vacuolate, but their identity is not lost. ALLEN (**1**) cites the formation of a spireme during the telophase of *Lilium canadense*. In *Pinus* and *Thuja* (LEWIS **24**) the identity of the chromosome is completely lost. NICHOLS (**31**) reports a similar condition in *Juniperus*.

HOMOTYPIC DIVISION.—In preparation for the second division the nuclear membrane disappears[2] and is succeeded by the formation of a spindle whose axis corresponds with the greater axis of the daughter nucleus. The daughter spireme is at first spread out on the spindle from pole to pole (fig. 21); later the chromatic mass contracts (fig. 22) and occupies a position at the equator of the spindle. At this time the spireme seems to be resolving into chromosomes (fig. 22). Throughout these stages there is no sign of a double filament; in fact the whole structure is indistinct. Figs. 23 and 24 show fully formed chromosomes which have split into daughter chromosomes. A side view of the spindle (figs. 23, 24) presents chromosomes apparently shaped like dumb-bells. A comparison of the above mentioned figures with fig. 25, a polar view of the equatorial plate, explains the actual condition. The daughter chromosomes are paired in the form of V's; the open ends of the V's are turned outward, the arms of the V's are nearly at right angles to the axis of the spindle. The appearance of the chromosomes of figs. 23 and 24, as described above, is due to the fact that only the tips of the chromosomes at the open ends of the V's can be seen; the seeming connection between the chromosome tips is occasioned by an indistinct view of the apices of the V's.

The separation of the daughter chromosomes as in the first division is not simultaneous. Fig. 23 shows a chromosome well on its way toward one pole before its sister chromosome, or the

[2] LAWSON in a recent paper (**23**) claims that the nuclear membrane does not break down or collapse as was formerly supposed to be the case. On the contrary, it behaves as a semi-permeable plasmatic membrane. Changes in the quantity and form of the chromatin previous to the metaphase are apparently accompanied by a change in the osmotic relations of the karyolymph. As a result of this there is a gradual decrease in the volume of the nuclear vacuole until the nuclear membrane closes in about the chromosomes; each chromosome becomes a single osmotic system.

other chromosomes, have moved far from the equatorial plate. Fig. 26 also shows chromosomes in the anaphase lagging behind at the equator, while the majority have nearly reached the poles.

CHROMOSOME NUMBER.—The metaphase of the second division represents the most favorable opportunity for the chromosome count. The chromosome number, however, was not determined with any finality. In diakinesis the chromosomes, though few in number, are so large that they obscure each other. A comparison of the counts attempted during diakinesis and the second metaphase places the haploid number of chromosomes at either 12 or 13.

Postsynapsis in the megasporangium

The difficulty of obtaining maturation stages of the megaspore mother cell discouraged a study of meiosis in the pistillate material. Of the many slides prepared, the majority showed synapsis; in addition only a few postsynaptic stages were procured. The nuclei of the megaspore mother cells are larger than those of the microspore mother cells, and when desirable stages are found they are exceedingly favorable for study.

The first stage noted after synapsis represents the nucleus as containing a mass of loosely interwoven filaments undivided and slightly beaded (fig. 28). The filaments thicken (fig. 29) and split longitudinally (fig. 30). Before the spireme breaks up into the bivalent chromosomes, it passes into strepsinema; the halves of the double filament draw apart, twist about, and cross each other (fig. 31). Attenuated portions of the spireme may be observed; transverse segmentation has begun and isolated pairs of chromosomes may be seen near the periphery of the nucleus.

In diakinesis the paired chromosomes occupy many positions with respect to each other, seldom lying strictly parallel. In fact, the description of diakinesis in the staminate loculus applies here. In fig. 33 several of the chromosomes offer a trace of a split, probably a precocious homotypic fission. In the microspore mother cells this split was not observed until the metaphase. This homotypic fission is described as occurring earlier in plants studied by the metasyndetists (MOTTIER, LEWIS, FARMER, DAVIS,

and others). MOTTIER (29) finds gemini in *Tradescantia* having double paired segments. This doubling is supposedly due to the reappearance of a split in the spireme which is believed to be a genuine homotypic fission and hence comparable to the split found in the paired segments of gemini in *Smilax*.

Mode of reduction

The mode of reduction in *Smilax herbacea* is essentially parasyndetic, though the procedure in the prophase seems to depart from the method described by GRÉGOIRE (17) for parasyndesis. According to GRÉGOIRE, the chromatin in the prophase takes the form of thin paired filaments (leptonema) which fuse (zygonema), shorten and thicken (pachynema), and again separate (strepsinema). In *Smilax* the chromatin in the prophase is distributed in granules, which are frequently seen in pairs. That which takes place between this condition and the spireme is obscured by synapsis. The construction and relative arrangement of the chromosomes in the spireme can be inferred only from subsequent behavior. After synapsis, two longitudinal splits occur; the first appears early in the spireme and can be traced through strepsinema to diakinesis; the second split shows sometimes in the univalent halves of the gemini at diakinesis, but more often not until the metaphase. From this we may infer that the first doubling is a separation of previously paired elements and that the chromosomes or chromatic bodies are placed side by side in the spireme. The second doubling is a genuine fission.

Discussion

PERSISTENCE OF CHROMOSOMES.—Much of the cytological work of recent years has brought forward directly or indirectly the question of the persistence of chromosomes or of some smaller unit. The rediscovery of the work of MENDEL has given added impetus to the hope of finding a physical basis for heredity or the unit characters of MENDEL which, according to experiment, seem to pass from generation to generation inviolate. Opinions concerning the existence of this cytological unit necessarily vary. Some favor the idea of persistent chromosomes; some maintain that

the unit is smaller; while the observations of others imply the non-existence of a persistent chromatin body.

The work of OVERTON strongly supports the theory of chromosome persistence. In the resting somatic nuclei of *Thalictrum purpurascens* and *Calycanthus floridus* the chromosomes are represented by definite visible bodies, the prochromosomes (OVERTON **33, 34**). From his study of the nuclei in the pollen mother cells of *Campanula grandis*, *Helleborus foetidus*, *Thalictrum purpurascens*, and *Calycanthus floridus*, he draws the conclusion that the chromosomes never lose their identity in either somatic or germ nuclei. Even on the spireme the chromosome unit is distinctly visible. During interkineses (OVERTON **35**) of somatic mitoses progressive vacuolation and enlargement of the chromosomes take place, but the chromosome outline can always be traced. LAIBACH (**21**) in working on the Cruciferae finds that the chromosomes remain as clearly defined in the resting condition as during mitosis.

ROSENBERG (**36**), in the resting nucleus of the hybrid *Drosera longifolia*×*rotundifolia*, finds paired chromatic bodies that equal the number of somatic chromosomes. These he calls prochromosomes or centers of chromosome formation. DAVIS (**9**) described chromatic bodies in the nuclei shortly after the last division in the archesporium of *Oenothera gigas*, which he thinks probably are chromatin centers or prochromosomes.

On the other hand, the theory of nucleolar origin of chromosomes does not support the view of chromosome permanence. The author has already referred to the work of WAGER (**43**) and DARLING (**7**) describing the budding of chromosomes from the nucleolus. SHEPPARD (**38**) investigated the behavior of the nucleolus in *Hyacinthus*. In the spireme stage he found the nucleolus apparently being drawn out upon the chromatin threads by means of nucleolar pseudopodia connected with the chromatin threads. Here, as described, the chromatin does not originate entirely from the nucleolus. BERGHS (**3**) found large nucleoli in the somatic cells of *Spirogyra* which break up into bodies partly chromatic and partly achromatic equal to the number of chromosomes. A more detailed citation of this will occur later. Miss DIGBY (**11**) states that there is no relation between the number of

chromatic aggregations in the resting nucleus of *Galtonia candicans* and the number of chromosomes; moreover, in the telophase of the somatic divisions the chromosomes lose their identity, their centers dissolving and the chromosomes breaking into small portions.

Many cytologists compromise on a middle ground and assume that bodies which are smaller than the chromosomes and into which the chromosome is divisible, are the chromatic units. These have been styled as pangens (MOTTIER 29) or chromomeres (ALLEN 1, LEWIS 24). In the ordinary use of the above terms the pangen represents a smaller unit than the chromomere; in this connection the terms are used simply to designate a small chromatic body of no determined size. The chromosome represents a definite group of these units and is probably formed for the purpose of facilitating segregation and mitosis. ALLEN figures the actual union of chromatin granules in the spireme, with their subsequent separation. MOTTIER finds no evidence of prochromosomes but supports the theory of the individuality of pangens. LEWIS describes granules in the resting nucleus in excess of the number of chromosomes.

The differences among the above citations are not as serious as they might seem. By the adoption of a hypothetical unit smaller than the chromosome, it is not difficult to imagine that its appearance, whether alone or in close approximation to its fellows, might vary and vary much with the different species of plants studied. In the plants studied by OVERTON and LAIBACH the chromosomes may be said to pass from one stage to another always in definite uniform groups, the prochromosomes. We may say these bodies maintain their permanence because of an unchanging mutual attraction of the chromomeres in each chromosome. We may conceive of another condition in which the mutual attraction of the chromomeres in each chromosome group varies with the resting and active stages of the nucleus. Although ROSENBERG (36) found the somatic number of chromatin bodies in the resting nuclei of the hybrid *Drosera*, he considered them as chromatin centers about which chromatin units congregated in the prophase, always with the same relative arrangement. This theory advanced by ROSENBERG may be modified and extended to

cover a condition where no chromatin centers are visible, but in which the chromatin units, or small groups of units, arising from the fragmentation of a single chromosome, exert a mutual attraction and come together in a uniform body during the prophases.

It is somewhat more difficult to apply this conception to the cases of nucleolar origin of chromosomes. Although little is known about the nature and structure of the nucleolus, it seems plausible that the same relations between chromomeres may exist whether they are inclosed in a comparatively small body, the nucleolus, grouped in several small bodies, the chromosomes, or scattered in the nucleus.

An obstacle to the view of the persistence of either chromosomes or smaller chromatin units arises, however, when we consider the recent work of certain authors, such as that of Miss DIGBY (10) on *Galtonia candicans*, or that of GATES (16) on *Oenothera gigas*. Miss DIGBY describes a condition in *Galtonia* in which chromatin buds off from the nuclear framework, synaptic knot, or nucleolus, and passes into the cytoplasm or even into neighboring cells; these buds eventually disintegrate. Though Miss DIGBY implies that the parent nuclei develop normally, she does not describe their development beyond the spireme stage. However, she cites cases where entire loculi contained aborted pollen mother cells. GATES describes a similar phenomenon in *Oenothera gigas*. During the synaptic stage there is an extrusion of a part of the chromatic matter of the spireme into an adjoining cell; the extruded portion degenerates, but the nucleus from which it came behaves normally. CARRUTHERS (6), in a description of the cytology of *Helvella crispa*, states that there are extrusions of chromatin-like material from the poles of the nucleus, which disintegrate in the cytoplasm and take up nucleolar stains. GRIGGS (18) says, of the masses of chromatin in the nuclei of *Rhodochytrium*, a portion remains free and is cast into the cytoplasm or remains as beads on the spindle fibers, while the rest of the chromatin forms the chromosomes.

It is also not without interest to recall the condition of *Spirogyra* (BERGHS 3, MITZKEWITSCH 27) where a chromatic nucleolus during the anaphase shows tiny chromosomes enveloped in its mass; at the metaphase the nucleolus is divided and the halves move

toward the poles of the spindle where the nucleolar mass is resolved into large chromosomes. Upon decolorization, small portions, the size of the prophasic chromosomes, at the equatorial ends of the large chromosomes strongly retain the stain. The deeply stained portions of the large chromosomes BERGHS considers the "chromosomes veritables." In describing the larger bodies the word "chromosome" is used merely for convenience.

From the observations just noted, we may draw the conclusion that there are substances which are not chromatic in the sense of being the carriers of hereditary qualities, but which at some stages have the same appearance and pass through certain phases in closest proximity to the real chromosome. CARRUTHERS (6) suggests that the extruded bodies are masses of nutritive material for which the nucleus has no further use.

Conditions in *Smilax herbacea* do not support the theory of persistence of the chromosome as a physical unit, but of a smaller unit. The number of chromatin bodies in the microspore mother cell exceeds the number of somatic chromosomes; the same is true of the somatic nuclei of the nucellus. As these chromatin bodies vary in size, we consider that they are aggregates of units or chromomeres; also that the size of the chromomere aggregate varies with the number of units contained in it. Finally, during synapsis the chromomere aggregates form chromosomes according to a law of natural affinity. There is no evidence of a loss of chromatin or chromatin-like material.

PAIRING OF CHROMATIC ELEMENTS.—Closely connected with the theory of the persistence of chromatic units is the theory of the pairing of parental elements throughout the sporophytic phase. Before the theory of permanent, paired chromatic units was advanced, "pairing" was described only in connection with the prophase of the first meiotic division where parallel conjugation (parasynapsis or parasyndesis) of thin chromatic filaments was considered typical. A large group of cytologists now present a different mode of conjugation, namely, of the "end to end" type (telosynapsis or metasyndesis).

The difference between the two methods is not as important as it seems to be. Too much stress has been laid on the comparative

merits of parasyndesis and metasyndesis. Granted that the chromosomes are fully formed, so far as the arrangement of constituent parts is concerned, at the time of synapsis, there need be no difference in the ultimate result whether the homologous chromosomes appear in the spireme side by side or one ahead of the other; in either case the paired chromosomes are adjacent and are not prevented by their previous arrangement from exhibiting the same relations from diakinesis through succeeding stages. JORDAN (20) suggests that both parasynapsis and telosynapsis may occur in the same prophase; that is to say, the "end to end" arrangement of chromosomes in the spireme is frequently followed by a pronounced loop formation, resulting in a parallel approximation of chromosomes. On the other hand, parasynapsis may be followed by fusion of the ends of paired chromosomes (diakinesis). GATES (15) explains the appearance of both types of conjugation from a mechanical standpoint. According to his view short chromosomes are particularly adapted to telosynapsis, while long chromosomes are parasynaptic.

The extension of the theory of chromosome pairing to cover the entire sporophytic phase is supported by the observations of several investigators. STRASBURGER (39), in a study of the root tips of *Pisum*, found many cases where the chromosomes were grouped in pairs on the nuclear plate. He concludes that the parental chromosomes in the nuclei of the sporophyte generation do not form two separate groups, but that the homologous chromosomes occur in definite positions with respect to each other. He also figures a similar condition in an integument cell of the ovule of *Lilium Martagon* (STRASBURGER 40). Miss SYKES (42) describes a paired arrangement of chromatic elements in the somatic nuclei of *Hydrocharis Morsus-ranae* and *Bryonia dioica*. *Lychnis dioica* and *Sagittaria montevidensis* show fully formed chromosomes lying in pairs. OVERTON (34) states that, in the somatic nuclei of plants which he has studied, definite chromatic bodies were visible lying in pairs (*Campanula grandis*, *Helleborus foetidus*, *Thalictrum purpurascens*, *Calycanthus floridus*). BONNET (4), however, finds no satisfactory evidence of pairing in the diploid nuclei of the *Yucca*, although he finds definite chromosomes.

In plants which do not possess the persistent chromosome, the chance of observing the paired arrangement of smaller groups of chromatic units is much lessened. As mentioned early in this paper, the chromomeres or chromomere aggregates in either somatic or germ nuclei of *Smilax herbacea* frequently appear in pairs or double pairs. This seems to occur too often to be merely a coincidence, yet it could not be determined as a universal condition.

THE SEX DETERMINANT IN PLANTS.—Although no reference to the sex determinant has been made in the preceding pages, this study was first attempted with the hope of finding the idiochromosome in a dioecious plant. WILSON (44), in his studies of the determination of sex in insects, places the decisive sex factor in the sperm. Here he found one-half of the sperms each carrying one or more extra chromosomes or a chromosome unique in size. All cases which cannot be placed in the above groups he relegates to a group where there is no physical variation of the chromosomes in the sperm cells, but where one may presume a physiological variation.

Botanists have been unsuccessful in their efforts to find the idiochromosome. DARLING (7) in working on the sexual cells of *Acer Negundo* (staminate material) found that one daughter nucleus after the first division contained a secondary chromatin mass; after the second division, two of the granddaughter nuclei each contained one more chromatin mass than the other two. In the resting stage, however, all the nuclei looked alike. To these secondary masses DARLING attached a possible sexual significance. Miss SYKES (42) found the nuclei of both sexes of unisexual plants she had studied (*Hydrocharis Morsus-ranae, Bryonia dioica, Lychnis dioica, Mercurialis perennis, Sagittaria montevidensis, Cucurbita Pepo*) to be identical in the number and form of the chromosomes. STRASBURGER'S (41) efforts to find a structural basis for the determination of sex were rewarded with negative results. On the nuclear reduction plate of *Melandryum rubrum* he observed a pair of chromosomes much larger than the other gemini; this same condition appeared again on the homotypic spindle. Thus, one large chromosome was distributed to each of the tetraspores.

However, he found that, though the four pollen grains of each group usually agreed in size, there were groups in which two of the pollen grains were larger than the corresponding two. This possible indication of sex differentiation was destroyed when he found a like condition among the pollen grains of hermaphrodite plants (*Lychnis Flos-jovis*, *Silene fimbriata*).

In *Smilax herbacea* no trace of an idiochromosome was found. During the metaphase of the first division one frequently found a chromosome pair which anticipated the other gemini in separating and moving toward the poles (figs. 17, 18), but no differences were observed in the homotypic division or in the tetraspores. Moreover, precocious chromosome movement away from the equator during the heterotypic division has been reported by CARDIFF (5) in *Salomonia*, a hermaphrodite.

For the present, then, we may assume a physiological difference in the tetraspores of dioecious plants which has no physical manifestation.

Summary

1. The nuclei of the young microspore mother cells each contain several nucleoli of varying size. The nucleoli fuse during the prophase, forming one large nucleolus at synapsis. During the early prophase the nucleolus is provided with several "papillae." These doubtless represent small nucleolar bodies which also fuse with the larger nucleoli. The nucleolus usually has at least one papilla until its disappearance at the metaphase.

2. The chromatin in the young microspore mother cell occurs in the form of granules or chromomere aggregates (the chromomere is here considered a chromatic unit).

3. There is no presynaptic reticulum, leptonema, or zygonema. The chromatin granules are held in an indefinite linin mesh.

4. Synapsis is reached by a contraction of the linin-supporting structure drawing the chromatin granules together.

5. The chromatic elements emerge from synapsis in the form of a spireme which soon becomes double.

6. The spireme shortens and thickens. Transverse segmentation of the spireme results in the formation of long paired chromo-

somes which continue to shorten and thicken, producing the characteristic gemini of diakinesis.

7. The separation of homologous chromosomes at the metaphase proceeds as usual. At this stage the chromosomes frequently show a split preparatory for the second division.

8. At the telophase a nuclear membrane appears. During interkinesis the chromatin is in the form of a band, apparently wound about the periphery of the nucleus. The band seems to be split or slightly vacuolate.

9. With the formation of the spindle of the second division the nuclear membrane disappears and the chromatic band resolves into chromosomes.

10. At the homotypic metaphase the longitudinal halves of the chromosomes separate.

11. The method of reduction in *Smilax herbacea* essentially coincides with the "hétérohoméotypique" scheme of GRÉGOIRE.

12. The persistent chromatic body in *Smilax* is a smaller unit than the chromosome.

13. The pairing of chromatic bodies was observed in the prophase, but not as a universal phenomenon. The same condition was evident in the nuclei of the nucellus.

14. An effort to find a sex determinant in *Smilax* was futile.

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EXPLANATION OF PLATES IV–VI

Microspore mother cells

FIGS. 1 and 2.—Young spore mother cell; nucleoli fusing; ×2400.

FIG. 3.—Same as fig. 1; nucleolar "papillae"; ×2400.

FIG. 4.—Young spore mother cell; paired chromatin bodies; ×2400.

FIG. 5.—Spore mother cell with one papillate nucleolus; chromatin bodies in pairs; ×2400.

FIG. 6.—Early synapsis; ×1725.

FIG. 7.—Synapsis; ×1725.

FIGS. 8, 9.—Late synapsis; ×1725.

FIG. 10.—Spireme; ×1725.

FIG. 11.—Early spireme stage; ×2400.

FIG. 12.—Spireme; ×2400.

FIG. 13.—Late spireme; beginning of transverse segmentation of spireme; ×2400.

FIG. 14.—Strepsinema; ×2400.

FIG. 14*a*.—Pair of homologous chromosomes; same stage as fig. 14; ×2400.

FIG. 15.—Diakinesis; ×2400.

FIGS. 16–18.—Metaphase; ×2400.

FIG. 19.—Late anaphase; ×2400.

FIG. 20.—Interkinesis; ×2400.

FIGS. 21–26.—Homotypic phases.

FIG. 21.—Prophase; ×1725.

FIG. 22.—Late prophase; ×1725.

FIG. 23.—Metaphase; ×1725.

FIG. 24.—Metaphase; ×1725.

FIG. 25.—Metaphase; polar view of equatorial plate; ×1725.

FIG. 26.—Late anaphase; ×2400.

FIG. 27.—Nucleus from somatic cell of young ovule; ×2400.

Megaspore mother cells

FIG. 28.—Early spireme stage; ×2400.

FIG. 29.—Spireme; ×2400.

FIG. 30.—Spireme; later stage than fig. 29; ×2400

FIG. 31.—Strepsinema; ×2400.

FIGS. 32, 33.—Diakinesis; ×2400.